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(54) Title: CHEMILUMINESCENT SIGNAL ENHANCEMENT

(57) Abstract

The present invention provides a method for generating an enhanced chemiluminescent signal from an acridinium sulfonamide compound, the method comprising the step of contacting the acridinium sulfonamide compound with a trigger solution in the presence of an enhancer to obtain a chemiluminescent signal which is stronger than would otherwise be generated in the absence of said enhancer. The enhancer comprises at least one member selected from the group consisting of nonionic surfactants, zwitterionic surfactants, and anionic surfactants.

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CHEMILUMINESCENT SIGNAL ENHANCEMENT

Background of the Invention

1. Field of the Invention

The present invention relates to immunoassays utilizing chemiluminescent compounds and, more particularly, to the use of surfactants for enhancing the chemiluminescent signal of acridinium sulfonamides.

2. Background of the Invention

Immunoassays which employ a chemiluminescent label as the signal generating compound are known. For example, chemiluminescence generation and detection in immunoassays is disclosed in W. R. Seitz, "Immunoassay Labels Based on Chemiluminescence and Bioluminescence," Clinical Biochemistry, 17:120-126 (1984). The use of acridinium esters as labels for immunoassays and subsequent generation of short-lived chemiluminescence signals from these labels is disclosed in I. Weeks, et al., "Acridinium Esters as Highly Specific Activity Labels in Immunoassays," Clinical Chemistry, 19:1474-1478 (1984).

Methods of increasing the intensity of a chemiluminescent signal generated by acridinium esters and certain other labels in an immunoassay are also known in the art. For example, U.S. Patent No. 4,959,182 describes a method for amplifying the chemiluminescent signal generated from alkaline phosphatase-catalyzed 1,2-dioxetanes by the addition of a surfactant and a fluorescent compound attached to it. U.S. Patent No. 4,927,769 discloses use of certain types of surfactants for enhancing the chemiluminescent signal generated from acridinium ester conjugates. The method uses a first signal generation reagent under acidic conditions and, a second signal generating reagent to increase the pH and trigger the chemiluminescent signal. The acridinium esters require using this two stage process to efficiently emit the signal. This is a problem, however, in that antibody-conjugated acridinium esters are often unstable above

certain pH levels due to hydrolysis.

To avoid this problem, other chemiluminescent labels have been developed. For example, stable acridinium sulfonamides as labels for immunoassays is described in commonly-assigned published European Patent Application No. ~~273,115~~ of Mattingly et al. However, while acridinium sulfonamides are useful chemiluminescent reagents, there presently exists a need to enhance the generated chemiluminescent signal of these compounds in order to improve immunoassay sensitivity and efficiency.

Therefore, a general object of the present invention is to provide enhancement of the chemiluminescent signal of acridinium sulfonamides. Other objects will hereinafter become evident to those skilled in the art.

Summary of the Invention

The present invention concerns a method for generating an enhanced chemiluminescent signal from an acridinium sulfonamide compound. The method comprises the step of contacting the acridinium sulfonamide compound with a trigger solution in the presence of an enhancer to obtain a chemiluminescent signal which is about 1.5 to 8 times stronger than would otherwise be generated in the absence of the enhancer.

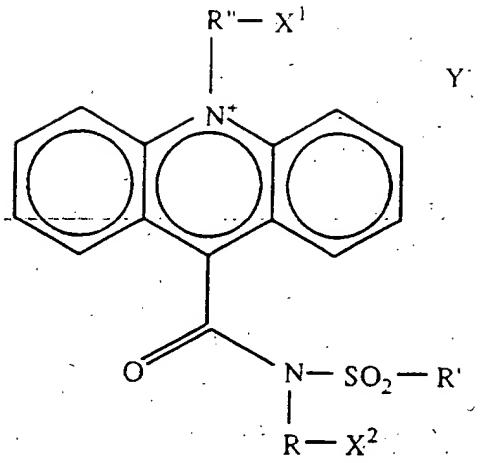
In a related aspect, the invention is a method for generating an enhanced chemiluminescent signal from an acridinium sulfonamide compound, the method comprising the step of contacting the acridinium sulfonamide compound with a trigger solution in the presence of an enhancer comprising at least one member selected from the group consisting of (i) nonionic surfactants, (ii) zwitterionic surfactants and (iii) anionic surfactants.

The invention further provides a trigger solution for generating a chemiluminescent signal from an acridinium sulfonamide compound. The trigger solution comprises an oxidant and an enhancer. The enhancer comprises a surfactant effective for increasing the intensity of the chemiluminescent signal generated by the acridinium sulfonamide compound.

The trigger solution of the invention, containing the oxidant and the enhancer, can be provided as a reagent in an immunoassay test kit in which the acridinium sulfonamide compound is included as another reagent. Accordingly, the invention is further directed to an immunoassay test kit having reagents which comprise (a) an acridinium sulfonamide compound; (b) a trigger solution separate from said acridinium sulfonamide; and (3) an enhancer comprising at least one surfactant selected from the group consisting of (i) nonionic surfactants, (ii) zwitterionic surfactants and (iii) anionic surfactants. The acridinium sulfonamide compound can be provided in the form of an acridinium sulfonamide-labeled conjugate for use in a variety of immunoassay formats. In a preferred embodiment of the test kit, made possible by our discovery that the enhancer of the present invention is stable in the trigger solution, the acridinium sulfonamide conjugate can be supplied as one reagent, and a trigger solution containing a mixture of the oxidant and the enhancer can be provided as a second reagent. Alternatively, the trigger solution and the enhancer can be supplied separately from one another. Further, the enhancer can be supplied in the form of a mixture with the acridinium sulfonamide compound.

Detailed Description

As used herein, the term "acridinium sulfonamide compound" means the chemiluminescent compounds identified by the formula:



wherein R, R', R", X^1, and X^2 are substituents which do not interfere with the chemiluminescent signal provided by such chemiluminescent compounds, with the proviso that R"-X^1 and R-X^2 may be independently hydrogen. More specifically, R and R" may be spacer arms and X^1 and X^2 may be independently members selected from the group consisting of hydrogen, carboxy, carboalkoxyl, carboxamido, carboaryloxy, cyano, carboximido, isocyanato, isothiocyanato, sulfo, sulfonyl halide, carbonyl halide, N-succinimidyloxycarbonyl and N-succinimidyloxysulfonyl. Y^- is an appropriate counterion, and may be selected from the group consisting of sulfate, alkylsulfate, halosulfate, haloborate, haloacetate, halophosphate, phosphate and halide. Preferably, the counterion is sulfate or halide.

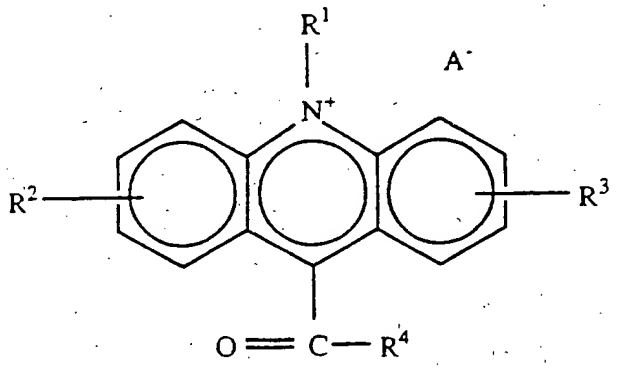
R, R', and R" may independently include a member selected from the group consisting of alkyl, alkylene, aryl, substituted alkyl, substituted alkylene, and substituted aryl groups, such that one or more hydrogens of said member can be replaced by an alkyl, aryl, alkylene, substituted alkyl, substituted alkylene, substituted aryl, alkoxy, aryloxy, halo, amino, protected amino, substituted amino hydroxy, protected hydroxy, oxo, thio, imino, mercapto or substituted mercapto group; or such that one or more carbon atoms of the member can be replaced by a heteroatom. The heteroatom may be selected from the group consisting of nitrogen, phosphorus, sulfur and oxygen.

R and R" independently may also be spacer arms of the

formula $-(CH_2)_n-$ where n = 0-50.

The preferred acridinium sulfonamide compounds for use in the present invention are 10-methyl-N-(2-carboxyethyl)-N-tosyl-9-acridinium carboxamide and 10-(3-sulfopropyl)-N-(2-carboxyethyl)-N-tosyl-9-acridinium carboxamide. The most preferred acridinium sulfonamide is 10-(3-sulfopropyl)-N-(2-carboxyethyl)-N-tosyl-9-acridinium carboxamide.

Also suitable for use in the present invention are the acridinium sulfonamide compounds referenced in Molz et al. European Patent Application No. 257,541 (published March 2, 1988) incorporated herein by reference. The acridinium sulfonamide compounds discussed in Molz et al. have the following general formula:



In which R^1 stands for hydrogen, an alkyl, alkenyl or alkynyl radical with 1 to 10 carbon atoms, a benzyl or aryl group, R^2 and R^3 stand for hydrogen, an alkyl group with 1 to 4 carbon atoms, a substituted or unsubstituted amino group, a carboxy, alkoxy, cyano, nitro group or halogen, R^4 represents a radical in which a sulfonamide group is bound directly to the carbonyl group via the nitrogen or a thioalkyl or thioaryl radical of formula II

$$-S \cdot X \cdot R^5 \qquad (II)$$

where X is a branched or unbranched aliphatic or aromatic group which may also contain heteroatoms, and R^5 is a reactive group which selectively under gentle conditions

can enter into a bond with amino, carboxy thiol or other functional groups in substances of biological interest, and A^- is an anion which does not impair chemiluminescence.

Preparation of the acridinium sulfonamide compounds useful in the present invention is disclosed in Mattingly et al. European Patent Application 273,115 published July 6, 1988, incorporated herein by reference. Molz et al. published European Patent Application No. 257,541 also discusses preparation of acridinium sulfonamides.

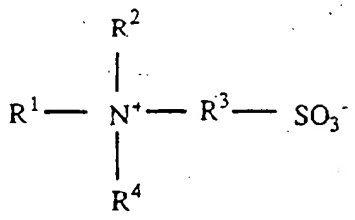
The acridinium sulfonamide can be oxidized by any oxidant which reacts with the acridinium sulfonamide to yield a product in an electronically excited state. As it returns to the ground state, this product releases energy in the form of light in a chemiluminescent reaction.

As used herein, a "trigger solution" means the solution containing the oxidant which initiates or catalyzes the chemiluminescent reaction. A preferred trigger solution comprises hydrogen peroxide in dilute alkali.

As used herein, the term "enhancer" means a reagent provided by the present invention that increases the total light emission of the chemiluminescent reaction and/or the signal to background noise ratio of the chemiluminescent reaction in comparison to that achieved by the acridinium sulfonamide in the absence of the enhancer. The enhancer is comprised of at least one member selected from the group consisting of nonionic, zwitterionic, and anionic surfactants.

Nonionic surfactants are discussed in Surfactant Science and Technology, by Drew Myers, VCH Publishers, Inc., N.Y., 1988, which is incorporated by reference herein. Preferred nonionic surfactants include polyoxyethylenated alkylenated alkylphenols, polyoxyethylenated straight-chain alcohols, polyoxyethylenated sorbitol esters, and alkanolamine-fatty acid condensates. Commercially available nonionic surfactants suitable for use with the present invention include TRITON X-100, TWEEN-20 and BRIJ-35.

Zwitterionic surfactants are also discussed in Myers, supra. Preferred zwitterionic surfactants are of the formula:



wherein R^1 is C_{14}-C_{16} aliphatic; R^2 and R^4 are methyl or ethyl; and R^3 is C_1-C_3 aliphatic. Other suitable zwitterionic surfactants include the formula wherein R^1 is C_8-C_{20} aliphatic; R^2, R^4, and R^3 are C_1-C_4 aliphatic. Commercially available zwitterionic surfactants include Tetradecylzwittergent, Hexadecylzwittergent and CHAPS.

Anionic surfactants are also discussed in Myers, supra. Preferred anionic surfactants include lithium dodecylsulfate, sodium dodecylsulfate and cholic acid.

We have found that the cationic surfactants Merquat and Cyastat, which are of the type disclosed in U.S. Patent No. 4,927,769, provide little or no enhancement of the acridinium sulfonamides.

Generally, enhancers suitable for use according to the present invention should be soluble in the reagents under the conditions in which the chemiluminescent reaction takes place. We have discovered that the surfactants prescribed for use as enhancers in the present invention enhance the chemiluminescent signal from acridinium sulfonamides such that the intensity of the signal is about 1.5 to 8 times greater, and preferably about 3 to 8 times greater than the signal intensity obtained in the absence of the enhancers. These results are unexpected because not all surfactants provide enhancement, much less the same degree thereof, in the acridinium sulfonamides. Moreover, the enhancement provided by the surfactants prescribed for use in the present invention, including without limitation, the degree of response, could not have been predicted from the enhancement observed in the case of acridinium esters. For

example, the cationic surfactants Merquat and Cyastat perform poorly or not at all as enhancers for the acridinium sulfonamide compounds used in the present invention.

Secondary reaction conditions which can affect the degree of signal enhancement include pH, temperature, and reagent concentration.

As described below, the preferred range for the enhancer present in an immunoassay chemiluminescent reaction is from about 0.25% to about 5% by weight, based on the total weight of the solution in which the chemiluminescent reaction takes place (i.e., the solution containing the enhancer, the trigger solution and the acridinium sulfonamide compound). More preferably, the enhancer should be present in such solution in an amount of about 2% by weight.

By amplifying the signal generated by acridinium sulfonamide compounds, the enhancers of the present invention facilitate determination of the presence of an acridinium sulfonamide in a test sample. A particularly useful application of the present invention involves determination of an acridinium sulfonamide conjugate in an immunoassay. Thus, an immunoassay test sample suspected of containing an analyte can be contacted with (i) the acridinium sulfonamide compound (i.e., a conjugate of the acridinium sulfonamide with an antigen, hapten, antibody, nucleic acid, etc.); (ii) the trigger solution; and (iii) an enhancer according to the invention, so as to generate an enhanced signal. The trigger and the enhancer can be combined before addition to the acridinium sulfonamide. The acridinium sulfonamide solution and the trigger/enhancer solution can be brought in contact with an analyte either sequentially or simultaneously. Techniques for preparing acridinium sulfonamide conjugates for use in immunoassays are described in Mattingly et al. published European Patent Application 273,115, incorporated by reference herein.

The present invention can be employed in various heterogeneous and homogeneous immunoassay system formats

known in the art. Such immunoassay system formats include, but are not intended to be limited to, competitive and immunometric techniques. Generally, such immunoassay systems depend upon the ability of a binding member, such as, for example, an immunoglobulin (i.e., a whole antibody or fragment thereof) to bind to a specific analyte from a test sample, wherein a labeled reagent comprising a binding member labeled with a chemiluminescent compound, such as the acridinium sulfonamide described herein, is employed to determine the extent of binding. Typically, the extent of binding in such immunoassay system formats is determined by the amount of the chemiluminescent compound present in the labeled reagent which either has or has not participated in a binding reaction with the analyte, wherein the signal which is generated by the chemiluminescent compound as described herein is detected and correlated to the amount of analyte present in the test sample. The test sample can be any material suspected of containing the analyte. The test sample can be used directly as obtained from the source or following a pretreatment to modify the character of the sample.

Homogeneous immunoassays typically are performed in a competitive immunoassay format involving a competition between an analyte from a test sample and a labeled reagent for a limited number of receptor binding sites on an antibody to the analyte. The labeled reagent comprises the analyte or analyte-analog labeled with a chemiluminescent compound wherein the concentration of analyte in the test sample determines the amount of the labeled reagent that will specifically bind to the antibody. The amount of the labeled reagent-antibody conjugate produced by such binding may be quantitatively measured and is inversely proportional to the amount of analyte present in the test sample.

Heterogeneous immunoassay formats involve a labeled reagent or tracer comprising an analyte, analyte-analog, or an antibody thereto, labeled with a chemiluminescent compound. The assay involves formation of a free species and a bound species. In order to correlate the amount of

tracer in one of such species to the amount of analyte present in the test sample, the free species must first be separated from the bound species, which can be accomplished according to methods known in the art employing solid phase materials for the direct immobilization of one of the binding participants in the binding reaction, such as the antibody, analyte-analog, or analyte, wherein one of the binding participants is immobilized on a solid phase material, such as a test tube, beads, particles, microparticles or a matrix of fibrous material, and the like, according to methods known in the art. The solid phase materials can be any solid material to which a binding participant can be immobilized and include, but are not intended to be limited to, beads, magnetic particles, paramagnetic particles, microparticles or macro particles, test tubes, and microtiter plates. Such solid phase materials can be made from synthetic materials, naturally occurring materials, or naturally occurring materials which have been synthetically modified, and include, but are not intended to be limited to, cellulose materials, such as paper, cellulose and cellulose derivatives such as cellulose acetate and nitrocellulose; fiberglass; naturally occurring cloth such as cotton; synthetic cloth such as nylon; porous gels, such as silica, agarose, dextran, and gelatin; porous fibrous matrixes; starch based materials, such as cross-linked dextran chains; ceramic materials; olefin or thermoplastic materials including polyvinyl chloride, polyethylene, polyvinyl acetate, polyamide, polycarbonate, polystyrene, copolymers of vinyl acetate and vinyl chloride, combinations of polyvinyl chloride-silica; and the like.

Heterogeneous immunoassays can be performed in a competitive immunoassay format wherein, for example, the antibody can be immobilized to a solid phase material whereby upon separation, the signal generated by the chemiluminescent compound of the bound or free species can be detected and correlated to the amount of analyte present in the test sample. Another form of a heterogeneous immunoassay employing a solid phase material is referred to

as a sandwich immunoassay, which involves contacting a test sample containing, for example, an antigen with a protein such as an antibody or another substance capable of binding the antigen, and which is immobilized on a solid phase material. The solid phase material typically is treated with a second antigen or antibody which has been labeled with a chemiluminescent compound. The second antigen or antibody then becomes bound to the corresponding antigen or antibody on the solid phase material and the signal generated by the chemiluminescent compound in the bound or the free species can be detected and correlated to the amount of analyte present in the test sample.

As used herein, the term "test sample" means any sample derived from any biological source, such as a physiological fluid, including, blood, saliva, ocular lens fluid, cerebral spinal fluid, sweat, urine, milk, ascites fluid, mucous, synovial fluid, peritoneal fluid, amniotic fluid or the like. The test sample can be pretreated prior to use, such as preparing plasma from blood, diluting viscous fluids, or the like; methods of treatment can involve filtration, distillation, concentration, inactivation of interfering components, and the addition of reagents. Besides physiological fluids, other liquid samples can be used such as water, food products and the like for the performance of environmental or food production assays. In addition, a solid material suspected of containing the analyte can be used as the test sample. In some instances it may be beneficial to modify a solid test sample to form a liquid medium or to release the analyte. The analyte can be any compound or composition to be detected or measured and which has at least one epitope or binding site.

As used herein, the term "analyte" means any substance for which there exists a naturally occurring binding member or for which a binding member can be prepared. Analytes include, but are not limited to, toxins, organic compounds, proteins, peptides, microorganisms, amino acids, nucleic acids, hormones, steroids, vitamins, drugs (including those administered for therapeutic purposes as well as those

administered for illicit purposes), virus particles and metabolites of or antibodies to any of the above substances. In particular, such analytes include, but are not intended to be limited to, ferritin; creatinine kinase MB (CK-MB); digoxin; phenytoin; phenobarbital; carbamazepine; vancomycin; gentamicin, theophylline; valproic acid; quinidine; luteinizing hormone (LH); follicle stimulating hormone (FSH); estradiol; progesterone; IgE antibodies; vitamin B12 micro-globulin; glycated hemoglobin (Gly. Hb); cortisol; digitoxin; N-acetylprocainamide (NAPA); procainamide; antibodies to rubella, such as rubella-IgG and rubella-IgM; antibodies to toxoplasmosis, such as toxoplasmosis IgG (Toxo-IgG) and toxoplasmosis IgM (Toxo-IgM); testosterone; salicylates; acetaminophen; hepatitis B virus surface antigen (HBsAg); antibodies to hepatitis B core antigen, such as anti-hepatitis B core antigen IgG and IgM (Anti-HBC); human immune deficiency virus 1 and 2 (HIV-1 and HIV-2); human T-cell leukemia virus 1 and 2 (HTLV-1 and HTLV-2); hepatitis Be antigen (HBeAg); antibodies to hepatitis Be antigen (Anti-HBe); thyroid stimulating hormone (TSH); total thyroxine (total T4); free thyroxine (free T4); total triiodothyronine (Total T3); free triiodothyronine (Free T3); carcinoembryoic antigen (CEA); and alpha fetal protein (AFP). Drugs of abuse and controlled substances include, but are not intended to be limited to, amphetamine; methamphetamine; barbiturates such as amobarbital, secobarbital, pentobarbital, phenobarbital, and barbital; benzodiazepines such as librium and valium; cannabinoids such as hashish and marijuana; cocaine; fentanyl; LSD; methaqualone; opiates such as heroin, morphine, codeine, hydromorphone, hydrocodone, methadone, oxycodone, oxymorphone and opium; phencyclidine; and propoxyhene. The term analyte also includes any antigenic substances, haptens, antibodies, macromolecules and combinations thereof. The foregoing is not intended to be a limiting definition.

As used herein, the term "analyte-analog" means any substance which cross-reacts with an analyte-specific

binding member, although it may do so to a greater or lesser extent than does the analyte itself. The analyte-analog can include a modified analyte as well as a fragmented or synthetic portion of the analyte molecule, so long as the analyte-analog has at least one epitope site in common with the analyte of interest. An example of an analyte-analog is a synthetic peptide sequence which duplicates at least one epitope of the whole-molecule analyte so that the analyte-analog can bind to an analyte-specific binding member. The binding member is a member of the binding pair, i.e., two different molecules wherein one of the molecules specifically binds to the second molecule through chemical or physical means. In addition to antigen and antibody binding pair members, other binding pairs include, as examples without limitation, biotin and avidin, carbohydrates and lectins, complementary nucleotide sequences, complementary peptide sequences, effector and receptor molecules, enzyme cofactors and enzymes, enzyme inhibitors and enzymes, a peptide sequence and an antibody specific for the sequence or the entire protein, polymeric acids and bases, dyes and protein binders, peptides and specific protein binders (e.g., ribonuclease, S-peptide and ribonuclease S-protein), and the like. Furthermore, binding pairs can include members that are analogs of the original binding member, for example, an analyte-analog or a binding member made by a recombinant techniques or molecular engineering. If the binding member is an immunoreactant it can be, for example, a monoclonal or polyclonal antibody, a recombinant protein or recombinant antibody, a chimeric antibody, a mixture(s) or fragment(s) of the foregoing, as well as a preparation of such antibodies, peptides and nucleotides for which suitability for use as binding members is well known to those skilled in the art.

The above-described invention is useful for enhancing the chemiluminescent signal provided by acridinium sulfonamides. Immunoassays assays in which the invention can be used include, by way of example, and not by way of limitation, assays for thyroid hormones, cancer markers,

viral antigens or their antibodies, therapeutic drugs, etc.

The invention provides a simpler, more sensitive and more convenient chemiluminescent immunoassay than previously described. Simplification results in that the enhancer reagent can be added in a single stage for an effective chemiluminescent immunoassay.

A further benefit of the invention is the attainment of markedly improved assay efficiency. Improved efficiency makes it possible to achieve desired assay results using less acridinium sulfonamide conjugate. This benefit results in a substantial reduction in assay cost without sacrificing assay sensitivity.

The following Examples are set forth for purposes of illustration and are not intended to limit the present invention.

EXAMPLE 1

Materials

Antibody to human thyroid stimulating hormone (h-TSH) was labeled with 10-(3-sulfopropyl)-N-(2-carboxyethyl)-N-tosyl-9-acridinium carboxamide. For convenience, this sulfopropyl substituted acridinium sulfonamide will hereafter be referred to as "acridinium sulfonamide (sulfopropyl)." Similarly, prostate specific antigen (PSA) goat antibody was separately labeled with 10-methyl-N-(2-carboxyethyl)-N-tosyl-9-acridinium carboxamide and with 10-(3-sulfopropyl)-N-(2-carboxyethyl)-N-tosyl-9-acridinium carboxamide. This methyl substituted acridinium sulfonamide will hereafter be referred to as "acridinium sulfonamide (methyl)." The preparation of the acridinium (methyl)- and acridinium (sulfopropyl)-labeled antibodies was carried out as described in commonly assigned Mattingly et al. published European Patent Application 273,115, incorporated herein by reference.

The enhancers tested included: Merquat (a cationic surfactant dimethyldiallylammonium chloride) purchased from Calgon Corporation, Pittsburgh, PA; TWEEN-20 (sorbitan monooleate polyoxyethylene) and BRIJ-35 (lauryl alcohol

ether polyoxyethylene) both obtained from Fisher Scientific, Fairlawn, NJ; TRITON X-100 (a-[4-(1,1,3,3,-Tetramethyl-butyl)phenyl]-w-hydroxypoly(oxy-1,2-ethanediyl), CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propane-sulfonate) and LDS (lithium dodecylsulfate) obtained from Sigma Chemical Company of St. Louis, MO; SDS (sodium dodecylsulfate) obtained from Bio-Rad Laboratories, Richmond, CA; CYASTAT (a cationic surfactant 3-lauramidopropyltrimethyl ammoniummethylsulfate) purchased from American Cyanamide Company, Charlotte, NC; and cholic acid purchased from Aldrich Chemical Company of Milwaukee, WI.

A Model LB 9501 Luminometer was obtained from Laboratorium Berthold, Wildbad, Germany.

The trigger was an alkaline peroxide solution of 0.25 N NaOH containing 0.6% hydrogen peroxide (H_2O_2) which also contained 0.01% antifoam agent. The antifoam agent was Antifoam C Emulsion Sigma No. A-8011 obtained from Sigma Chemical Company, St. Louis, MO. We have not observed any effect of the antifoam agent upon signal intensity output.

Method

The acridinium sulfonamide conjugate was diluted with distilled water to give approximately 250,000 counts and was added in the amount of 100 ul to a vial and placed in the luminometer. While the vial was in the measuring position, 300 ul of trigger containing 1% of the selected enhancer was injected into the vial. A control sample was also measured which used a trigger containing no enhancer. The light emitted was measured in the luminometer for two seconds.

Results

Table 1 shows the ratios of the signal intensity enhancement exhibited by the chemiluminescent reaction of the acridinium sulfonamide (sulfopropyl)-labeled h-TSH antibody, the acridinium sulfonamide (sulfopropyl)-labeled PSA antibody, and the acridinium sulfonamide (methyl)-labeled PSA antibody over the two second measuring time in

the presence and absence of CHAPS, Merquat (cationic), SDS, LDS, TWEEN-20, BRIJ-35, TRITON X-100, CYASTAT (cationic) and cholic acid.

TABLE 1

Ratio of Signal Intensity Enhancement

	Acridinium Sulfonamide Antibody Conjugates		
	Sulfopropyl-Labeled h-TSH	Sulfopropyl-Labeled PSA	Methyl-Labeled PSA
Control	1.0	1.0	1.0
CHAPS	1.6	1.7	1.5
Merquat	0.9	0.9	1.0
SDS	1.2	1.3	3.0
LDS	1.3	1.3	3.0
TWEEN-20	1.2	1.2	2.1
Brij-35	1.6	1.6	2.3
TRITON X-100	2.3	2.3	3.1
CYASTAT	1.8	1.6	1.5
cholic acid	1.5	1.4	1.7

EXAMPLE 2

Materials

The enhancers tested included TRITON X-100, SDS, BRIJ-35 and TWEEN-20, as well as Hexadecylzwittergent (N-hexadecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate) and Tetradecylzwittergent (N-tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate which were both obtained from Sigma Chemical Company. Folate labeled with acridinium ester was obtained from CIBA Corning Diagnostics, Corp., Medfield, MA. Acridinium sulfonamide (sulfopropyl)-labeled h-TSH antibody was prepared, as described in Example 1. Similarly, acridinium sulfonamide (methyl)-labeled h-TSH antibody was prepared. The trigger reagent was as described in Example 1.

Method

The acridinium sulfonamide conjugates were diluted with distilled water to produce approximately 7,000 counts when triggered without an enhancer. 50 ul of the diluted conjugate and 50 ul of 0.03 N H_2SO_4 were added to a vial. The acid was necessary to obtain light emission from the ester conjugate. The triggering process was as described in Example 1.

Results

Table 2 shows the ratios of the signal intensity enhancement exhibited by the chemiluminescent reaction of ester-labeled folate, the acridinium sulfonamide (sulfopropyl)-labeled h-TSH antibody (Ab), and the acridinium sulfonamide (methyl)-labeled h-TSH antibody (Ab) over the two second measuring time in the presence and absence of TRITON X-100, SDS, Brij-35, TWEEN-20, Hexadecylzwittergent and Tetradecylzwittergent.

TABLE 2

Ratio of Signal Intensity Enhancement

Enhancer	Folate Ester	h-TSH Ab Sulfopropyl	h-TSH Ab Methyl
Control	1.0	1.0	1.0
TRITON X-100	10.4	4.0	6.4
SDS	2.6	1.0	2.8
Brij-35	3.8	1.8	3.0
TWEEN-20	2.6	1.1	2.3
Hexadecyl-zwittergent	10.1	4.7	6.1
Tetradecyl-zwittergent	23.0	5.6	7.6

EXAMPLE 3

Materials

Magnetic microparticles were coated with PSA monoclonal antibody using EDAC (1-ethyl-3(3-dimethyl aminopropyl)carbodiimide hydrochloride) coupling as

previously described in U.S. Patent 3,857,931 (Hagar, H. 1974) which is hereby incorporated by reference. They were then diluted to a concentration of approximately 0.15% solids.

A standard containing 100 ng/ml PSA (Abbott Diagnostics, Abbott Park, IL) was used. Acridinium sulfonamide (sulfopropyl)-labeled goat PSA antibody described in Example 1 was used to test two enhancers. The enhancers included TRITON X-100 and Tetradecylzwittergent. The wash solution used was a microparticle enzyme immunoassay (MEIA) diluent buffer available from Abbott Diagnostics. The trigger solution used is described in Example 1.

Method

The following protocol was then used for a PSA assay. A suspension containing 50 ul of the magnetic microparticle solid-phase and 50 ul of the PSA standard was incubated at 37 °C for ten minutes. The microparticles were washed two times by subjecting them to a magnetic field. The microparticle pellet was resuspended in 150 ul of the acridinium sulfonamide-labeled PSA antibody solution. This suspension was incubated at 37 °C for 20 minutes after which the microparticles were subjected to a magnetic field and washed four times. The washed microparticles were then mixed with 400 ul of distilled water and placed in the luminometer in contact with trigger solution whereupon the signal was read as described in Example 1.

Trigger solutions containing 0.0%, 0.5%, 1.0% and 2.0% TRITON X-100 and Tetradecylzwittergent were used to determine the effect of enhancer concentration on light emission.

Results

Table 3 shows the effect of TRITON X-100 and Tetradecylzwittergent enhancer concentration on the light emission from the PSA assay. The effect is demonstrated by the ratios of signal intensity enhancement.

TABLE 3

Ratio of Signal Intensity Enhancement

	Enhancer Concentration			
Enhancer	0%	0.5%	1.0%	2.0%
TRITON X-100	1.0	1.68	2.28	3.50
Tetradecyl-zwittergent	1.0	3.72	3.94	4.46

EXAMPLE 4

Materials

Magnetic microparticles were coated as described in Example 3, with PSA antibody, hepatitis B surface antigen (HBsAg) antibody, h-TSH antibody, and triiodothyronine antigen (T_3) antibody. The calibrators used were as follows: the calibrator for HBsAg was recalcified human plasma spiked to 0.5 ng/ml with surface antigen, PSA as described in Example 3, and the h-TSH and T_3 calibrators are both from Abbott Diagnostics.

As described in Example 1, h-TSH antibody was labeled with acridinium sulfonamide (sulfopropyl). Similarly, the PSA antibody, HBsAg antibody and T3 were also separately labeled with the acridinium sulfonamide (sulfopropyl).

The enhancers tested included Tetradecylzwittergent and TRITON X-100. The trigger reagent was an alkaline peroxide solution of 0.25 N NaOH containing 0.45% H_2O_2 which also contained 0.01% antifoam agent as described in Example 1.

Method

The optimal range for the enhancer concentration and the trigger was determined for four different assays. The same standard assay protocol described in Example 3 was used, with the following exceptions. The sample size for the HBsAg and h-TSH assays was 200 ul instead of 50 ul. The second incubation period for the T_3 assay was shortened to ten instead of twenty minutes. The concentrations of

the enhancers used were 0.5%, 2.3%, and 4.0%.

Results

The Relative Light Units (RLU's) of the chemiluminescent reaction of the four assays with varying concentrations of two enhancers are exhibited in Table 4.

TABLE 4

Effect of TRITON X-100 on Chemiluminescence (RLUs)

Calibrator	Assay	0.5%	2.3%	4.0%
0.5 ng/ml	HBsAg	440	788	810
100 ng/ml	PSA	173,000	270,000	293,000
100 ulU/ml	h-TSH	1,108,000	1,790,000	1,940,000
0 pg/ml	T_3	25,000	43,000	46,000

Effect of Tetradecylzwittergent on Chemiluminescence (RLUs)

Calibrator	Assay	0.5%	2.3%	4.0%
0.5 ng/ml	HBsAg	860	940	1030
100 ng/ml	PSA	283,000	342,000	327,000
100 ulU/ml	h-TSH	1,850,000	1,914,000	1,946,000
0 pg/ml	T_3	36,000	37,000	39,000

EXAMPLE 5

Materials

The acridinium sulfonamide conjugate solutions, magnetic microparticles and calibrators were prepared in the same manner described in Example 4. The enhancers tested included Tetradecylzwittergent and TRITON X-100. The trigger was an alkaline peroxide solution of 0.25 N NaOH containing 0.3% H_2O_2 which also contained 0.01% antifoam agent and 2% of the enhancer.

Method

The same standard protocol for the assay described in Example 3 was used. The only difference was that the assays were performed with the conjugate concentration at 0.25X, 0.5X and 1X the concentration chosen for the

unenhanced assay.

Results

Table 5 shows the RLU's of the chemiluminescent reaction of sulfopropyl-labeled conjugates of h-TSH, HBsAg and PSA antibodies and T_3 at varying concentrations over the two second measuring time in the presence of TRITON X-100 and Tetradecylzwittergent. A control contained no enhancer.

TABLE 5

Effect of TRITON X-100 on Chemiluminescence (RLUs)

Assay	Control	0.25X	0.5X	1.0X
HBsAg	266	390	500	610
PSA	86,000	84,000	154,000	267,000
h-TSH	600,900	668,000	1,064,000	1,562,000
T_3	10,800	12,400	21,000	31,000

Effect of Tetradecylzwittergent on Chemiluminescence (RLUs)

Assay	Control	0.25X	0.5X	1.0X
HBsAg	266	460	700	910
PSA	86,000	106,000	197,000	409,000
h-TSH	600,900	931,000	1,411,000	1,926,000
T_3	10,800	12,000	19,600	29,100

EXAMPLE 6

Materials

The effect of pH on the conjugates was tested by preparing the four different assays described in Example 4. The enhancer tested was TRITON X-100. The trigger was an alkaline peroxide solution of 0.25 N NaOH containing 0.3% H_2O_2 which also contained 0.01% antifoam agent and 2% of the enhancer.

Method

The same standard protocol for the assay described in Example 1 was used. The only difference was that the four assays were performed with the same trigger and the pH was

varied from 12.3 to 13.2.

Results

Table 6 shows the ratio of the trigger with the enhancer to the trigger without the enhancer at different pH values. The sulfopropyl acridinium sulfonamide conjugates of h-TSH, HBsAg and PSA antibodies and T_3 were measured over the two second time period in the presence and absence of 2% TRITON X-100.

TABLE 6

Ratio of Signal Intensity With & W/O Enhancer

pH	PSA	h-TSH	HBsAg	T_3
13.2	3.88	3.57	4.55	4.48
13.0	3.83	3.51	4.36	4.75
12.8	3.78	3.77	4.27	4.63
12.6	3.69	3.61	4.08	4.39
12.3	3.66	3.31	3.97	4.08

As a matter of convenience, the reagents necessary for practicing the chemiluminescence enhancement method of the present invention in an immunoassay can be provided in the form of a reagent kit where the reagents are in predetermined ratios, so as to optimize sensitivity of the assay in the range of interest. Wet or dry reagents may be used. If dry reagents are used they can be reconstituted prior to use to obtain a desired concentration of the reagents for a particular assay.

The reagents may be mixed with various ancillary materials such as members of the signal producing system, buffers, and the like. In a reagent kit according to the present invention, the trigger is kept separate from the acridinium sulfonamide. The enhancer may be kept separate from or added to either the trigger reagent or the acridinium sulfonamide reagent. The chemiluminescent reaction is then triggered by combining the trigger reagent with the acridinium sulfonamide.

While particular embodiments and applications of the

present invention have been illustrated and described, it is to be understood that the invention is not limited to the precise construction and compositions disclosed herein and that various modifications, changes, and variations which will be apparent to those skilled in the art may be made in the arrangement, operation, and details of construction of the invention disclosed herein without departing from the spirit and scope of the invention as defined in the appended claims.

WHAT IS CLAIMED IS:

1. A method for generating an enhanced chemiluminescent signal from an acridinium sulfonamide compound, the method comprising the step of contacting the acridinium sulfonamide compound with a trigger solution in the presence of an enhancer to obtain a chemiluminescent signal which is about 1.5 to 8 times stronger than would otherwise be generated in the absence of said enhancer.

2. The method of claim 1 wherein the enhancer comprises a surfactant.

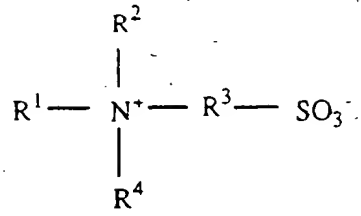
3. The method of claim 2 wherein the surfactant comprises at least one member selected from the group consisting of nonionic surfactants.

4. The method of claim 3 wherein the nonionic surfactant comprises at least one member selected from the group consisting of polyoxyethylenated alkylenated alkylphenols, polyoxyethylenated straight-chain alcohols, polyoxyethylenated sorbitol esters, and alkanolamine-fatty acid condensates.

5. The method of claim 4 wherein the surfactant is TRITON X-100.

6. The method of claim 2 wherein the surfactant comprises at least one member selected from the group consisting of zwitterionic surfactants.

7. The method of claim 6 wherein the zwitterionic surfactant has the formula



wherein R^1 is a C_8-C_{20} aliphatic moiety; and R^2, R^3 and R^4 are independently C_1-C_4 aliphatic moieties.

8. The method of claim 7 wherein the surfactant is at least one member selected from the group consisting of Hexadecylzwittergent, Tetradecylzwittergent and mixtures thereof.

9. The method of claim 2 wherein the surfactant is an anionic surfactant.

10. The method of claim 9 wherein the surfactant comprises at least one member selected from the group consisting of lithium dodecylsulfate and sodium dodecylsulfate.

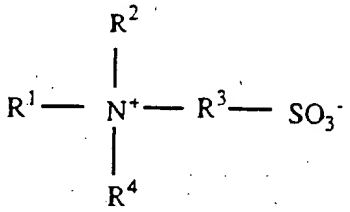
11. A method for generating an enhanced chemiluminescent signal from an acridinium sulfonamide compound, the method comprising the step of contacting the acridinium sulfonamide compound with a trigger solution in the presence of an enhancer comprising at least one member selected from the group consisting of (i) nonionic surfactants, (ii) zwitterionic surfactants and (iii) anionic surfactants.

12. The method of claim 11 wherein the surfactant is a nonionic surfactant comprising at least one member selected from the group consisting of polyoxyethylenated alkylenated alkylphenols, polyoxyethylenated straight-chain alcohols, polyoxyethylenated sorbitol esters, and alkanolamine-fatty acid condensates, and mixtures thereof.

13. The method of claim 12 wherein a chemiluminescent signal is generated which is about 1.5 to 8 times stronger than would otherwise be generated in the absence of said enhancer.

14. The method of claim 12 wherein the nonionic surfactant is TRITON X-100.

15. The method of claim 11 wherein the surfactant comprises a zwitterionic surfactant having the formula:



wherein R^1 is a C_8-C_{20} aliphatic moiety; and R^2, R^3 and R^4 are independently C_1-C_4 aliphatic moieties.

16. The method of claim 15 wherein a chemiluminescent signal is generated which is about 1.5 to 8 times stronger than would otherwise be generated in the absence of said enhancer.

17. The method of claim 15 wherein the surfactant comprises at least one member selected from the group consisting of Hexadecylzwittergent and Tetradecylzwittergent.

18. The method of claim 11 wherein the surfactant comprises an anionic surfactant selected from the group consisting of lithium dodecylsulfate, sodium dodecylsulfate and mixtures thereof.

19. The method of claim 18 wherein a chemiluminescent signal is generated which is about 1.5 to 8 times stronger than would otherwise be generated in the absence of said enhancer

20. The method of claim 11 wherein the acridinium sulfonamide compound comprises at least one member selected from the group consisting of 10-methyl-N-(2-carboxyethyl)-N-tosyl-9-acridinium carboxamide and 10-(3-sulfopropyl)-N-(2-carboxyethyl)-N-tosyl-9-acridinium carboxamide.

21. The method of claim 11 wherein the trigger solution comprises hydrogen peroxide in dilute alkali.

22. The method of claim 11 wherein the acridinium sulfonamide compound is present in the form of a conjugate with another compound.

23. The method of claim 11 wherein the acridinium sulfonamide comprises at least one member selected from the group consisting of 10-methyl-N-(2-carboxyethyl)-N-tosyl-9-acridinium carboxamide and 10-(3-sulfopropyl)-N-(2-carboxyethyl)-N-tosyl-9-acridinium carboxamide; the trigger solution comprises a compound capable of oxidizing the acridinium sulfonamide; and wherein said contacting step results in generation of a chemiluminescent signal which is about 1.5 to 8 times stronger than would otherwise be generated in the absence of said enhancer.

24. The method of claim 23 wherein the enhancer comprises TRITON X-100.

25. The method of claim 23 wherein the enhancer comprises at least one member selected from the group consisting of Hexadecylzwittergent and Tetradecylzwittergent.

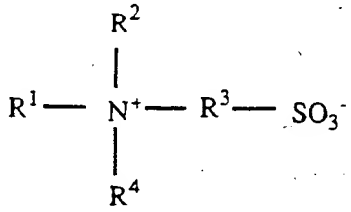
26. A trigger solution for generating a chemiluminescent signal from an acridinium sulfonamide compound, said solution comprising an oxidant and an enhancer, said enhancer comprising a surfactant effective for increasing the intensity of the chemiluminescent signal generated by the acridinium sulfonamide.

27. The trigger solution of claim 26 which, when contacted with an acridinium sulfonamide compound, results in generation of a chemiluminescent signal about 1.5 to 8 times stronger than would otherwise be generated upon contact of said trigger solution with said acridinium sulfonamide compound in the absence of said surfactant.

28. The trigger solution of claim 26 wherein the surfactant comprises at least one member selected from the group consisting of (i) nonionic surfactants, (ii) zwitterionic surfactants and (iii) anionic surfactants.

29. The trigger solution of claim 28 wherein the surfactant is a nonionic surfactant comprising at least one member selected from the group consisting of polyoxyethylenated alkylenated alkylphenols, polyoxyethylenated straight-chain alcohols, polyoxyethylenated sorbitol esters, and alkanolamine-fatty acid condensates, and mixtures thereof.

30. The trigger solution of claim 28 wherein the surfactant is a zwitterionic surfactant having the formula:



wherein R^1 is a C_8-C_{20} aliphatic moiety; and R^2, R^3 and R^4 are independently C_1-C_4 aliphatic moieties.

31. The trigger solution of claim 28 wherein the surfactant is an anionic surfactant comprising at least one member selected from the group consisting of lithium dodecylsulfate and sodium dodecylsulfate.

32. The trigger solution of claim 27 in contact with at least one acridinium sulfonamide compound selected from the group consisting of 10-methyl-N-(2-carboxyethyl)-N-tosyl-9-acridinium carboxamide and 10-(3-sulfopropyl)-N-(2-carboxyethyl)-N-tosyl-9-acridinium carboxamide.

33. An immunoassay test kit having reagents which comprise:

(a) an acridinium sulfonamide compound;

(b) a trigger solution separate from said acridinium sulfonamide compound; and

(c) an enhancer comprising at least one member selected from the group consisting of (i) nonionic surfactants, (ii) zwitterionic surfactants and (iii) anionic surfactants.

34. The test kit of claim 33 wherein the acridinium sulfonamide compound is at least one member selected from the group consisting of 10-methyl-N-(2-carboxyethyl)-N-tosyl-9-acridinium carboxamide and 10-(3-sulfopropyl)-N-(2-carboxyethyl)-N-tosyl-9-acridinium carboxamide.

35. The test kit of claim 34 wherein the trigger solution comprises hydrogen peroxide in a solution of dilute alkali.

36. The test kit of claim 34 wherein the surfactant comprises at least one member selected from the group consisting of TRITON X-100, TWEEN 20 and BRIJ-35.

37. The test kit of claim 34 wherein the surfactant comprises at least one member selected from the group consisting of Hexadecylzwittergent and Tetradecylzwittergent.

38. The test kit of claim 34 wherein the surfactant comprises at least one member selected from the group consisting of lithium dodecylsulfate, sodium dodecylsulfate and cholic acid.

39. The test kit of claim 33 wherein the enhancer is supplied in the form of a mixture with at least one other of said reagents.

40. The test kit of claim 39 wherein the mixture comprises the trigger solution.

41. The test kit of claim 39 wherein the mixture comprises the acridinium sulfonamide compound.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/07642

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/58 G01N33/533 G01N33/53 C07D219/04 C07D401/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,92 08979 (ABBOTT LABORATORIES) 29 May 1992 see page 3, line 4 - line 11; claim 6 see page 4, line 23 - line 32	26
A	see the whole document	1-25, 27-41
Y	US,A,4 927 769 (S.C.S. CHANG ET AL.) 22 May 1990 cited in the application see table 2	1-41
Y	EP,A,0 257 541 (HOECHST AG) 2 March 1988 cited in the application see the whole document	1-41

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
10 November 1995	29. 11. 95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Van Bohemen, C

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INTERNATIONAL SEARCH REPORT

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